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THE RADIOLOGICAL HEALTH ASPECTS OF AGENTS MODIFYING THE BIOLOGICAL EFFECTS OF RADIATION

Federation of America Societies for
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Bethesda, Maryland

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A STUDY OF

THE RADIOLOGICAL HEALTH ASPECTS OF AGENTS

MODIFYING THE BIOLOGICAL EFFECTS OF RADIATION

JUNE 1969

Prepared for

THE RADIOLOGICAL HEALTH STUDY SECTION
ENVIRONMENTAL CONTROL ADMINISTRATION
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HEALTH SERVICE
UNITED STATES PUBLIC HEALTH SERVICE
DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE

LIFE SCIENCES RESEARCH OFFICE
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I. FOREWORD

This is a technical report prepared for the Radiological Health Study Section, Bureau of Radiological Health, United States Public Health Service by the Staff of the Life Sciences Research Office, Office of Biomedical Studies, Federation of American Societies for Experimental Biology (FASEB). The text of the report reflects the opinions of an ad hoc study group that met at Beaumont, FASEB, on March 24 and 25, 1969. The report has been approved by the majority, not necessarily by all, of the participants.

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II. SUMMARY

This study summarizes and critically evaluates research on agents modifying the biological effects of radiation related to public health interests. The review includes the classes of agents that have been employed in the modification of the biological effects of radiation, their mechanisms of action, comparative effectiveness and toxicity, the methodology used in evaluating these agents, and their effectiveness against various radiation exposures. The conclusions of the report identify a general order of priority for future research.

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IV. CONCLUSIONS

The following conclusions identify a general order of priority for future research on the radiological health aspects of agents modifying the biological effects of radiation.

- Research on protection against the injurious biological effects of ionizing radiation has not emphasized protection against all levels and types of radiation that induce clinically evident effects related to public health interests. The chemical substances known to modify the biological effects of acute high level radiation exposures may afford some measure of protection against acute or repeated low level exposures to various radiations. However, there is no assurance that these compounds would be effective against low doses of radiation.

- A systematic screening program should be established to determine the effectiveness of these agents against low levels of radiation. Long-term experiments are necessary to understand the many facets of chemical protection against the delayed effects of low doses of radiation. Research studies on protective compounds will be costly, time-consuming, and require long range commitments. Acceptable screening techniques that measure the influence of protracted low doses of radiation are not available. Therefore, it is necessary first to develop adequate screening measures, and second to screen promising candidate compounds.

- Lack of knowledge of the basic mechanisms of radiation injury makes it difficult to develop a working hypothesis for the synthesis of improved radioprotective agents. The discovery of more effective and less toxic orally administered compounds depends upon this information, but screening programs will continue to exploit the existing opportunities.

- Promising studies have been conducted on cytochemical protein-bound sulfhydryl reactions and the intramolecular energy exchanges involved in the formation of disulfide bonds. The findings suggest that the measurement of these changes

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may lead to a better understanding of the mechanisms of action of radiation protective compounds. The newer physical chemical techniques that reveal precise protein conformational modifications should be exploited as a better way of relating cellular radiation injury to protection with the chemical agents.

• Relatively little attention has been given to the development of radiation effects modifying agents that encourage recovery processes following radiation injury. Current therapy of radiation injury is restricted to treatment of symptoms. More research is required on promising agents that may have therapeutic applications in treating the injurious effects of radiation.

• It may be possible to develop more satisfactory animal screening techniques for radiation effects modifying agents using the differential sensitivity of cells of the hematopoietic system. The crystalline lens of experimental animals has been used also as an in vivo test tissue. These objective screening measures may be most useful in measuring the effectiveness of compounds to protect against low levels of various types of radiation.

• Useful behavioral tests could be developed that reflect the biological effects of various kinds of radiation on the whole animal. The measures may include motor, sensory, or cognitive skills of animals using sophisticated behavioral techniques. The protective value of candidate compounds against low doses of radiation may be measured by these behavioral tests.

• Combinations of two or more agents that modify the biological effects of radiation should be studied in experimental animal models to determine their relative value against low doses of radiation and their relative toxicity. Combinations of protective compounds may prove to be useful in man both prophylactically and therapeutically.

• Inasmuch as clinically evident effects resulting from any type of radiation have public health interest, there is a continuing need to study the long-term effects as related to

intensity and the nature of the radiation. Epidemiological studies in various population groups will be useful in developing a data base. However, the research opportunities on the long-term radiation effects can be evolved best in experimental animal models.

- Greater research emphasis on agents modifying the biological effects of radiation for therapeutic use should be considered. The studies of tissue localization of anti-radiation agents should explore the opportunities for differential chemoprotection in normal and malignant tissues subjected to radiotherapy. The limited success in this field suggests that the degree of protection may be enhanced by further understanding of absorption, distribution, metabolism, and excretion of these compounds.

- Certain animals survive whole-body radiation doses that are lethal to nearly all similar members of the exposed group. The identification of this individual resistance will assist in the development of improved radiation modifying agents.

- Multiple environmental stressors on the irradiated subject are recognized as important in determining the degree of injury. The study of the combined effects of such factors as environmental extremes, physical and psychic stress, inanition, and pharmacotherapy must be made to assess their relative importance. These effects are extremely difficult to measure, are extraordinarily important, and will assist in determining the value of radioprotective agents under environmental conditions.

V. INTRODUCTION

This study was prepared for the Radiological Health Study Section, Bureau of Radiological Health, United States Public Health Service (USPHS), to review research on agents modifying the biological effects of radiation that may be applied to public health needs. The report is based on a technical review of the subject by a group of knowledgeable scientists actively participating in research on radiation biology and radiological health programs. The views expressed originated largely from the background of research and experience of the participants and the review and analysis of the subject by the Staff of the Life Sciences Research Office.

Historically, prophylactic and therapeutic regimens have focused upon the injury resulting from short duration, high dose exposure to ionizing radiation. The extent and severity of radiation-induced injury depends upon the characteristics of the radiation and the biological systems exposed. A number of chemical substances have been discovered that modify the biological effects of radiation. The major research emphasis on radiation protective agents has been injury prevention by administration of a compound prior to acute, whole-body, lethal doses of ionizing radiation. The expanding application of nuclear technology requires protection against the injurious biological effects of all levels of ionizing radiation.

Radiological health hazards are increased by manufacture and transportation of radioactive materials; the use of radiation for medical diagnosis and therapy; nuclear power operations; production and use of ionizing radiation in research and development; and the continued threat of radioactive fallout. The radiological health interests of the USPHS encompass research on the biological effects of radiation from these sources. The national program designed to prevent and control radiation hazards includes research to obtain the maximum benefits from the use of radiation with minimum risk to health. From a radiological health standpoint, prevention or modification of radiation effects such

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as cancer or germ cell mutation is at least as important as the prompt reduction of mortality or morbidity.

Effective radiation protective compounds could be used for radiation disaster victims as well as workers exposed to higher than acceptable levels of ionizing radiation; persons involved in rescue and decontamination procedures; individuals exposed to hazardous fallout from nuclear weapons, reactor or nuclear plant accidents; and patients receiving radiation for diagnosis and therapy.

Conceivably, compounds effective in some measure against relatively large, single radiation exposures might be used prophylactically against lower doses of radiation. Few compounds have been tested or evaluated in preventing injury from sub-lethal radiation exposures. The effectiveness of these compounds must be demonstrated against clinically evident effects of less than lethal radiation exposures in animals and man. Research advances of this character would have many public health implications.

VI. REVIEW DISCUSSIONS

A. BIOLOGICAL EFFECTS OF RADIATION

Different types of ionizing radiation produce biological effects that are qualitatively similar, but are indeed complex, because of the interrelationships of the quality, duration, and intensity of the radiation (1). Ionizing radiations induce reactions that injure the structural and chemical elements of the cell (2). Consideration of the radiological health aspects of agents that modify these injurious biological processes includes the entire spectrum of radiations.

Pihl and Eldjarn (3) considered ionizing radiation a pharmacologic agent. The pronounced dose-related effects, the specificity for cellular biochemical reactions, the lethality for cells, and the uniform distribution throughout tissues ironically resemble therapeutic drug actions. On the other hand, pharmacodynamically active compounds are specific in their actions while ionizing radiation might interfere with any biological function. It is remarkable that radiation produces minor alteration in one or relatively few molecules within the cell that may lead to serious deleterious effects. Lethal doses may result from only a small number of ionizations compared to the enormous number of molecules present in a normal cell. These facts suggest that target molecules of the cell are disrupted and this in turn leads to profound biological consequences (3). To unravel the nature of these molecular changes would be rewarding.

Most studies with the radiation effects modifying agents have been conducted with X- or gamma-rays. There are fundamental differences between the physical interactions of gamma rays, neutrons, and other types of radiations with matter. The differences are reflected in the magnitude of the biological effects of these forms of radiation. For example, many reports on the biological effects of neutron radiation deal with bacteria, and it is generally agreed that other organisms have different reactions to radiation (4).

Different radiations, even those exhibiting similar energy emission spectra, may induce quantitatively and qualitatively distinct radiation-induced biological changes.

Specific ionization is related to the average number of ion pairs formed per unit track length or LET (linear energy transfer). LET is often used as a basis for comparison of radiation effects in biological materials. In general, the higher the LET value, the greater the biological effectiveness of the radiation. Moreover, the relative biological effectiveness (RBE) compares the effectiveness of different radiations in producing a given change.

Radiations of high specific ionization tend to cause more biologic damage than low specific ionizing radiations. The RBE for various radiations generally increases with increasing LET, but the relation is not linear. Use of model systems permits development of hypotheses on the different effectiveness of densely ionizing radiations. It is conceivable that a similar relationship between the several forms of radiation may be manifest in chromosome aberrations. High-LET radiation effects on the chromosomes may not be repaired as readily as those of low-LET radiation. However, it is not known if these strands can be "fully repaired" once they are broken. This is one of the recognized hazards of interpreting chromosome aberrations as an estimate of radiation injury (5).

Biological phenomena related to the nature, duration, and intensity of radiation usually involve the difficult concepts of dose-rate and fractionated or divided doses. The latter factor is important because the living cell is constantly undergoing metabolic processes, growth, repair, and regeneration. A non-fatal dose of radiation will permit some degree of repair; repeated doses will be fatal to cells, tissues, and eventually the organism only if there is not adequate time for repair or if some secondary injurious process develops (6, 7, 8, 9, 10). The biologic effects of all radiations with respect to the rate of recovery of mammalian organisms following doses of varying intensity and duration should be explored in greater detail. Many of these factors have been

reviewed and broad outlines of research needs have been established (6, 11, 12, 13, 14, 15).

The concept of a threshold dose of radiation is debatable. This concept implies that there is some level of irradiation that does not produce any clinically evident effects. Jones (16) indicated the inappropriateness of the threshold theory of radiation effect, especially when applied to such phenomena as induction of cancer, cell killing, and life-shortening. Knowledge of these long-term radiation dose-effects is usually based on small mammal experiments and it is impossible to extrapolate the resulting data to man. The only data on man of low-level chronic radiation exposure are the studies of life-shortening in American radiologists and other medical specialists (17, 18). However, no relationship between mortality and exposure to ionizing radiation was observed for New England dentists during the period 1921 - 1960 (19). In any event, it seems reasonably proper to consider any radiation as deleterious, and although direct proof is lacking, radiation exposure should be presumed as life-shortening (20). Moreover, the risk of thyroid neoplasms after irradiation in childhood, as studied by Hempelmann (25) showed a dose response over a wide dose range (20 to 1125 rad). While the conclusive proof of lack of injury is unobtainable at the present time, the evidence strongly suggests and prudence dictates the desirability of as low a dose of radiation exposure as possible over the lifetime of the person.

The severity of the acute radiation syndrome in man following whole-body irradiation depends on the dose, dose-rate and fractionation, dose distribution to the body, and the degree of individual susceptibility. In general, any exposure to radiation resulting in clinically evident effects, whenever manifest, is deemed of paramount importance and constitutes the primary radiological health interest. For example, non-lethal doses of approximately 100 to 250 R may be of therapeutic significance; doses of approximately 10 to 100 R may produce illness but not death; somatic and genetic effects might occur after acute radiation exposure doses of approximately 10 to 25 R, or low doses administered over long time periods; at approximately 10 R there may be no overt

symptoms, but radiation does affect sensitive cells or cellular components.

The present stringent rules for protection of workers in radiation laboratories set the maximum permissible dose of whole-body penetrating radiation at about 250 rad spread over a working career; about 5 rad per year (21). Exposure of an individual in the population-at-large should be about one-tenth that for occupational purposes; or about 0.5 rad per year (22). The downward trend in permissible levels of radiation exposure reflects the recognized hazards of the lower doses of radiation-induced biological effects.

It is estimated that the typical radiation dosage from natural sources is about 0.10 to 0.13 rad per year. In some areas of the world, in parts of India and Brazil, the natural radiation exposure may be ten times higher. Comar (23) suggested that it would be interesting to know the consequences of such higher dosages on the relative health of the populations involved. However, studies are thus far incomplete and inconclusive.

The long-term effects of radiation may be equated to nonspecific insults to the body that mimic in some ways the effects of aging. In addition to inducing malignancies, radiation may produce other long-term effects that lead to shortening of the life span. The injurious radiation dose may be small depending upon the susceptibility of the tissues, as in the case of cancer induction in children following prenatal exposure to X-rays. The fetus may be more susceptible to certain forms of radiation-induced cancer than the adult, possibly because the progeny cells are in the embryonic formative stage of development and are more likely to generate tumors or mutations. In children whose thyroid gland cells are proliferating rapidly, the cells injured by radiation develop into neoplasms (24). The damaged thyroid cells are then able to proliferate for many cell generations. Conversely, in the normal adult gland conditions are unfavorable for this expression of cellular damage induced by radiation because parenchymal cells divide only infrequently (25). In the adult, the target cell may die and subsequently be discarded. Latent tumor virus activation by irradiation of mice that

harbor the virus has been demonstrated. The incidence of this causative factor for tumors in man is unknown.

It is impossible to give an accurate tabulation of the relative sensitivity of the different cells and tissues of the mammalian body to ionizing radiation. The selective action of radiation on different parts of the cell and the observation that radiation sensitivity is greatest for those cells with the highest mitotic rate is recognized. However, there are numerous factors such as oxygen tension, phase of mitotic cycle, genetic gackground, cell age, and metabolic state that influence radiation sensitivity (7, 20). The changes brought about by radiation exposure of the body may be caused by direct effects on the cell (scopal), or as a result of damage at some locus remote from the affected cell (abscopal). Adequate explanation of these changes is an unresolved problem of radiation biology.

The delayed somatic effects of ionizing radiation are difficult to study because many months or years are required to adequately accumulate the data. In addition, it is difficult, if not impossible, to relate the late effects to radiation exposure alone because many events of potentially equal significance will occur in the life of the individual. However, a few reports have indicated that radiation effects modifying agents will prolong the life of animals or prevent the development of neoplasms, cataracts, or nephrosclerosis characteristic of late effects of radiation exposure (26). Several measures including hypoxia or anti-radiation agents prolong the life of irradiated mice (27, 28, 29) and provide some measure of protection against the incidence of cancer in rats (30). For example, young adult female mice exposed to X-irradiation (350 R) were treated prior to radiation with a radioprotective (S, 2-aminoethylisothiurea) (29). The 60-day survivors were observed until death and necropsies were performed. Although the administered compound had a marked protective effect against early lethality, it was less effective in protecting against the death of the long-term survivors. The radioprotective inhibited only the development of thymic lymphomas. In addition, graying of the fur was reduced, but the incidence of nephrosclerosis was not changed. The lens opacities that developed in all aging mice,

including the controls, were increased in severity by radiation, and the radioprotective did not prevent the development of cataracts in these animals. In general, protection against the chronic effects of ionizing radiation was not as pronounced as that obtained for acute lethal effects.

Radiation-induced cataract is a late effect of radiation. Numerous reports have described the nature of human cataract as a complication of radiotherapeutic exposure of the lens (3, 20). In the adult the latent period may be 10 years or more, while the developing eye of infants and children is more prone to cataract formation.

Summaries of foreign publications, especially from the Russian literature have been reported (88, 89). Radioprotective substances for spaceflight, late effects of radiation exposure, and the recent development of the radiation protective agents and their evaluation are included. In general, the compounds that have been studied, the methods of testing, and the research approaches as described in these publications are similar to those outlined in this report.

B. AGENTS MODIFYING THE BIOLOGICAL EFFECTS OF RADIATION

Chemical Classes. Radioprotective agents* are defined as chemical compounds that decrease the biological effects of ionizing radiation when administered shortly before radiation exposure. These agents administered prior to radiation exposure are more effective in preventing the death of animals than the same substances given post-irradiation. The usual concept of the radioprotective chemical is a ubiquitously distributed "energy trap" that protects the cells of the animal body against penetrating ionizing radiation (31). However, it is difficult to envisage a nontoxic, reactive molecule of this character that would retain its selective radiation absorbing potency in equilibrium with the dynamic energy exchanges of the living cell. There are few therapeutic agents that will reverse radiation effects. By contrast, substances are known that potentiate rather than attenuate the effects of radiation (32).

A number of substances have been discovered to exhibit protective effects against whole-body irradiation of experimental animals. Numerous reviews on these chemical protective agents have been published (4, 31, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45). None of these compounds have been used prophylactically for radiation protection in man. The most promising include cysteine, MEA, AET, GSH, and WR 2721 (Table 1). With the exception of WR 2721, these aminothiols contain the mercapto group (sulfhydryl, -SH) with not more than 3 carbon atoms separating the amino and thiol moieties. Among the sulfur-free compounds, the most widely studied effective radioprotectants are 5-HT and PAPP.

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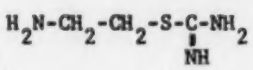
* Throughout the text of this report, the following terms are used interchangeably: radioprotective agents, radiation effects modifying agents, anti-radiation agents, radiation protectors, radiation protective agents, chemical protective agents, chemical protectors, radioprotectors, radioprotectives, radioprotectants, and compounds that afford radiation protection.

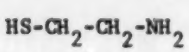
TABLE 1

RADIOPROTECTIVE CHEMICALS

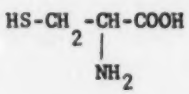
AET — S,2-aminoethylisothiurea



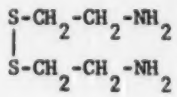
MEA — β-mercaptoethylamine
(cysteamine or 2-aminoethanethiol)



cysteine — β-mercaptoalanine



cystamine — 2,2'-dithiobis(ethylamine)



MEG — 2-mercaptoethylguanidine

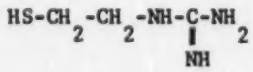
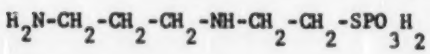
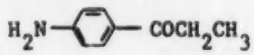


TABLE 1 (continued)

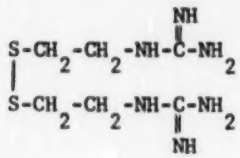
WR 2721* S-2-(3-aminopropylamino)ethylphosphorothioate



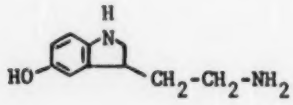
PAPP p-aminopropiophenone



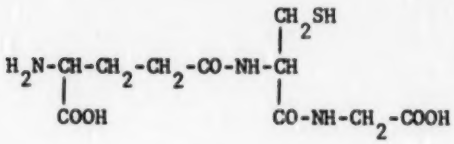
GED guanidoethyldisulfide



5HT 5-hydroxytryptamine (serotonin)



GSH γ-L-glutamyl-L-cysteinylglycine (glutathione)



* Walter Reed compound 2721

The research procedures developed in the Walter Reed Army Institute of Research program for effective and safe anti-radiation agents were reviewed in 1966 (14). This research program was designed to screen chemicals for their ability to prevent 50% radiation-induced lethality of mice in 30 days ($LD_{50/30\ day}$). Those agents that appear effective are given a secondary evaluation in the rodent to determine the duration of action and a more precise dose reduction factor (DRF) is calculated. Eventually, promising agents are evaluated in larger animals, dog, swine, or monkey. A core problem in this research has been to reduce the toxicity of effective compounds and to develop agents that could be used in man. Recently these studies have been declassified and will be published.

Thousands of compounds have been screened and only a few have been found worthy of toxicity testing in man. Some compounds provide approximately a twofold protection in relatively large oral doses. Acute, whole-body exposure of mice to nearly pure neutron radiation has disclosed that a similar pattern of protection can be achieved by prior administration of agents known to protect against gamma rays. Unfortunately, there has been relatively little study of protection against neutron radiation.

At the present level of research effort, continued synthesis and screening would likely produce even better compounds. This process is expensive, time-consuming, and involves a considerable measure of chance. Unfortunately, at the present time it is not known if those agents already developed would be useful in protecting against the clinically evident radiation effects that are of greatest interest in the radiological health program.

Mechanisms of Action. The several classes of chemical compounds that have been found to provide radiation protection exert their effects by different mechanisms. The greater effectiveness of two or more dissimilar compounds administered simultaneously presumably reflects such a difference. However, there is no clear understanding of the mode of action of these compounds or why one agent

is superior to another of the same general chemical category. Detailed studies of the biochemical actions of these substances at the cellular or tissue level are essential to elucidate their mechanisms of action.

The following mechanisms have been proposed to explain the effectiveness of agents that modify the biological effects of radiation:

- Formation of mixed disulfides;
- Induction of hypoxia by lowering the oxygen tension of tissues by one of several mechanisms;
- Scavenging free radicals to prevent radiation interaction with vital cell systems;
- Donation of hydrogen atoms to permit rapid repair of the target molecules within the cell;
- Chelation of metal ions essential for enzyme functions; and
- Energy traps for ionizing radiation to spare more vital molecular functions.

In general, chemical protective agents appear to reduce the "effective" radiation impinging upon the cell. The response of the animal to single-dose, whole-body radiation after administration of the protective compound simulates the effects of a lower radiation dose. In effect, the radiation dose is lowered and thus a dose reduction factor (DRF) can be calculated for any radiation protective compound.

Sulfhydryl compounds that have been studied extensively are believed to reduce radiation damage by several mechanisms including free radical scavenging, repair of damage of cellular elements, and by their capacity to form mixed disulfides(1, 4, 46, 47).

The thiols and disulfides are the best protectors of tissues and whole animals against gamma radiation. It is

believed, based largely on electron spin resonance (ESR) studies, that the highly reactive free radicals formed by radiolysis of water react with the molecules of the sulfhydryl moiety to prevent the interaction of cellular proteins with the energic free radical (4). For example, in irradiated salmon sperm nuclei (48) MEA, in the presence of oxygen, prevents the cross-linking of deoxyribonucleic acid (DNA) following gamma ray exposure. The protective agent donates a hydrogen atom to the radical restoring the original state. In the presence of MEA the rate of disappearance of free radicals, as measured by ESR, associated with the irradiated DNA, was increased with a corresponding increase in the free radical signal formed from MEA.

More precise and detailed knowledge is required concerning the cytochemical events associated with protein-bound sulfhydryl reactions and the energy exchanges in the formation of disulfide compounds in the normal cell. Obviously, the cellular biochemistry of these substances is related to the mechanism of action of the sulfhydryl radiation protective compounds. Bacq (4) suggested a survey of the various forms of known free and bound sulfhydryl and disulfide compounds be made to more critically investigate their reactions with radioprotectors. This is a logical step to make in the exploration for chemical information on radiation protection at the cellular level. Intimately associated with chemical exchange of the glutathione-cysteine type are the more subtle energy changes within intracellular elements, mitochondria, endoplasmic reticulum, ribosomes, nucleus, nucleolus, and the plasma membrane. Changes in macromolecular conformations induced by radiation may be inhibited by the radioprotectors. These morphologic modifications are closely related to the composition of the amino acids comprising the protein structures, including the thiol peptides.

One general mechanism is universally recognized as significant in radiation injury and protection. If the oxygen tension of the tissues is reduced by any mechanism such as reduced blood flow, binding of hemoglobin, or reduced oxygen saturation of the blood, the degree of injury produced by the immediate effects of the radiation will be lessened. Carbon

monoxide, vasoconstrictors, and tissue or central nervous system respiratory depressants that produce hypoxia are poor, but effective, radiation protectors.

Based on the concept that an ideal radiation protector is a molecule that can exchange both electrons and hydrogen atoms easily without itself becoming dissociated, Shimazu and Tappel (49) studied DL-selenomethionine and DL-selenocystine as model compounds. Selenoamino acids presumably should be superior protectors because the ionization potential and bond energy of selenium compounds are smaller than their corresponding sulfur compounds (49). In in vitro test systems the selenoamino acids proved to be better protectors than the analogous sulfur amino acids and sulfhydryl compounds. The protective effects of these compounds in animals have not been reported. However, phenylselenol was found to be inactive in mice (4).

The protective action of the aminothiols, e.g., cysteine and MEA, is thought to depend on intracellular mechanisms that control thiol-disulfide systems preventing the formation of potentially toxic disulfides.

The anti-radiation effect of PAPP appears to depend on its action in opposing the injurious role of oxygen in radiation damage. PAPP administration induces formation of methemoglobin; an overdose results in cyanosis and anoxia. However, there is no clear evidence that the effectiveness of PAPP depends on the formation of methemoglobin.

The radioprotective ability of 5-HT may be explained by the production of tissue hypoxia. A correlation exists between the decrease in cellular oxygen tension and the protective response. Serotonin (5-HT) reduces splenic oxygen tension in mice but is no longer protective if pretreated animals are irradiated under high oxygen pressure. Serotonin is effective in mice and rats but ineffective in the dog.

Combined Protective Agents. Combinations of several agents may afford greater radiation protection presumably because each substance acts in an independent manner and the effects may be additive. Treatment with two or more dissimilar protective compounds has proved successful in preventing the deaths of animals from single, acute radiation exposures (50, 51, 52, 53, 54, 55). Various combinations of 5-HT, MEA, AET, histamine, PAPP, dimethyl sulfoxide, and cystamine have been investigated (56). Mixtures of several radioprotectors increased the degree of protection beyond 30 days compared with the substances given separately. For example, the DRF for AET and 5-HT in combination was 2.3, while individually the DRF for each compound was 1.7. Diminished toxicity of such compounds as MEA and AET was observed, probably because lower doses of each compound were administered. In this study, the most effective combination was GSH + AET + 5-HT + cysteine + MEA with a DRF of 2.8. When supplemented by treatment with isogenic bone marrow after irradiation, the DRF increased to $\geq$ 3.7. Complete chemical protection against all types of radiation effects should not be anticipated unless appropriate therapy is instituted and maintained.

In another series of studies, 5-HT plus MEA or AET provided protection for mice against 800 R and 1100 R but not against 1400 R total body irradiation (53). When the radioprotective chemicals were given to mice before 800 R, 1100 R, and 1400 R, 95%, 92%, and 50% of the animals respectively, lived for 30 days. On day 36, survivors were again given the same chemical mixture and radiation exposures. It was found that 97%, 93%, and 77% of these mice respectively, survived the second radiation exposure for 30 days (54). Isologous bone marrow administered post-irradiation permitted a 91% thirty-day survival at 1700 R and 52% survival at 2000 R (51). Similar protection was obtained against supra-lethal doses of X-rays to dogs by the combined administration of PAPP, AET, and 5-HT prior to irradiation (52).

Sulfhydryl protection might be augmented by agents that produce hypoxia, vasoconstriction, central nervous system stimulation, and histaminic responses (4, 41, 57).

In general, while the experimental findings have been encouraging, the basis for these tests is empirical. Effective use of combining radioprotectors with pharmacologic agents will await a better understanding of their synergistic actions.

Relatively little attention has been given to developing radiation effects modifying agents that encourage the recovery processes following radiation injury. Therapy of radiation injury has embraced treatment of specific tissues or body systems, but no successful clinical technique includes the use of a drug or chemical substance to "antidote" the fundamental intracellular changes. However, to study the enhancement of "repair" it is necessary to know the causes of injury and the nature of the post-irradiation changes that may be modified. These changes presumably would be at the molecular, enzymic level of the protoplasm or membrane of the cell. It is noteworthy that bacteria and some other single-cell organisms are inherently capable of significant post-irradiation repair. The administration of thymus gland or spleen extracts (58) has been reported to enhance the early return of erythropoiesis after radiation exposure. Splenic extracts produced 94% survival in mice receiving $LD_{100/20\ day}$ X-ray doses when the extracts were injected daily for five days post-irradiation (59). Their value in post-irradiation treatment is currently unresolved and the subject requires study in greater detail.

The effectiveness of a radiation protective agent is influenced by absorption, distribution, metabolism, and excretion. In the treatment of cancer patients with ionizing radiation, the poor vascularity of solid tumors can be a therapeutic advantage. For this reason, radiation protective compounds may exhibit differential distribution and enhance the value of therapeutic radiation. This possibility was considered in explaining the action of WR 2721 in protecting normal tissues to a greater degree than a transplantable mammary mouse tumor against X-irradiation (60). A broad study of the tissue distribution and metabolism of all radioprotective agents would be valuable. The detection and quantitative determination of extremely low concentrations of biologically significant compounds can be made (61). This information may assist in determining the mechanisms of

action, metabolic products, and toxic effects of these agents.

Toxicity. A core problem in research on radioprotectives is to reduce the toxicity of effective compounds to man. The comparative effectiveness of anti-radiation compounds involves the measurement of toxicity of those substances that have prophylactic or therapeutic merits. There are limited opportunities for such toxicity testing in the irradiated man. Certain of these compounds have potential value in some disease states and this has assisted in obtaining clinical appraisal of the toxicity. Other therapeutic virtues of radiation effects modifying agents advantageously facilitate and justify prolonged and expensive toxicologic evaluations.

So few effective radiation protective compounds are available, and the challenge is potentially so rewarding, that there is a tacit acceptance of a greater degree of toxicity than might be demanded of ordinary therapeutic agents. These toxic effects would be more readily acceptable in nuclear disasters. In any event, if a compound gave good protection, side effects or toxicity would be considered tolerable. On the other hand, the biological effects of long-term, low-level radiation exposure may be so mild as to be preferable to the toxic effects of chronic administration of protective agents.

The toxicity problem is well illustrated by MEA and cysteine. Maximally tolerated doses of each afford the same degree of radioprotection, but MEA is more toxic than cysteine. Therefore, the two compounds are not therapeutically equivalent on a milligram basis. In the dog MEA causes side effects of salivation, vomiting, diarrhea, agitation, ataxia, tonic and clonic convulsions, and increased depth and rate of respiration. Irradiation following AET administration has been reported to increase the incidence of lymphoma in mice. Toxic responses of this latter character would not be acceptable. On the other hand, the mild symptoms of nausea, allergic reactions, mild hypotension, and similar reactions that are not life-threatening may be considered a low price to pay for a high order of radiation protection.

C. EVALUATION OF CANDIDATE AGENTS

Dose Reduction Factor. The most widely used measure of protection is the comparison of the dose-mortality curves of treated versus untreated irradiated animals (usually mice). The mathematical calculation of the increased tolerance to the radiation owing to the test agent is made by dividing the $LD_{50/30\ day}$ value of the treated animals by the $LD_{50/30\ day}$ figure for the untreated animals. This is expressed as the dose reduction factor (DRF). For example, if the $LD_{50/30\ day}$ dose for untreated control animals is 500 R, and equal lethality is found in the animals treated with the protective agent at 1000 R, the DRF is 2. The most effective compounds that have been discovered have DRF values of over 2. The experimental variables in DRF tests are great, and rigorous standardization is mandatory.

There have been many questions about the interpretations of the lethal dose-mouse survival test. Elaborate studies have been conducted to improve this screening measure, but no acceptable standard model has been developed. The test method does detect potentially useful compounds and these in turn may be evaluated in larger animals for acute and chronic toxicity. Most investigators agree that there is poor correlation between the mammalian lethal test systems and bacterial or tissue culture screening methods. Little is known of the relevance of these methods to the problem of modifying clinically evident radiation effects in man.

Individual Variations. Research workers studying the radiation protective agents have observed that many variables influence the radiation sensitivity of experimental animals. Such factors as diet, e.g., sulfur containing compounds, fats, or body hormone inhibiting substances, acclimatization to cold, hypothermia, and corticosteroids modify the sensitivity of the test animals (4). Presumably, physical, dietary, or physiological factors will also influence the susceptibility of man to radiation.

The phenomenon of individual variability is significant in the radiological health aspects of radiation effects modifying agents. Relatively little attention has been directed to the subject of the high degree of variability observed in different species, including man, exposed to different levels of radiation. The observation has been made repeatedly that some individual animals survive exposures to whole-body radiation doses that are lethal to nearly all similar members of the group. There is no adequate explanation for this fact and unknown factors must be influencing the lethality effects of the radiation exposure. A proper understanding of these unknown influences may provide useful clues to the development of improved radiation effects modifying agents.

Compounds that modify radiation effects should not enhance bacterial invasion, accentuate stress responses, or interfere with wound healing. Animal models have been used to study the combined effects of these insults to the animal and radiation injury. For example, experimental surgical wounds will increase the mortality of whole-body X-irradiated rats (62, 63, 64) or decrease the mortality from gamma rays, depending upon the timing of wounding and irradiation (65). Irradiated, wounded rats heal in a manner similar to non-irradiated animals if some radioprotective compound (MEA, 5-HT, WR 2721) is administered prior to radiation exposure. Combination-effect studies of this type should be made under a greater variety of experimental conditions: inanition, environmental, physical, and psychic stresses, and in combination with therapeutic agents.

Rodent lethality, organ weights, cataract formation, hematologic changes, and survival of bacterial or tissue culture cells can be measured objectively. However, the applicability of these measures to other than high dose exposure has not been studied. Obviously, improved screening methods are desirable for evaluating agents that modify the biological effects of radiation, including low-dose, long-term exposure. Although there is interest in the subject from a public health standpoint, the number of published reports is limited. The somatic effects of cataract formation or tumor production by low dose radiation offer possibilities for testing compounds against low intensity or prolonged

radiation exposure. The crystalline lens of experimental animals has been used as an in vivo test tissue to objectively measure the effects of radiation exposure. Eyes of the mouse, rat, guinea pig, rabbit, cat, dog, and monkey have been irradiated with X-rays, neutrons, deuterons, alpha particles, and beta rays in single and repeated doses (20). There appears to be no significant qualitative difference between species of animal or types of radiation. Radiation-induced hematologic changes have been used as early indicators of the degree of radiation injury. The hematopoietic system is extremely sensitive to radiation. Therefore, hematologic changes should be a most promising index to denote the effectiveness of protective agents against low radiation doses. The cellular elements of the blood are not uniformly radiosensitive and hence provide a discriminatory means of measuring agents capable of protecting against damaging effects of ionizing radiation. If these indices could be standardized, the hematopoietic changes could be used as a screening method for protective compounds. Little research effort has been expended in these latter fields in the study of radiation effects modifying agents.

Behavioral Studies. One novel approach to the subject is provided by the quantitated measure of animal behavioral changes induced by low doses of radiation (66, 67, 68, 69). A potential advantage of tests of this type is that they do not require lethal doses of radiation. Because animal behavioral studies presumably permit this estimation, they have considerable interest at the present time. However, radiation effects modifying agents themselves could influence these behavior changes and should be investigated.

Behavioral studies have shown that after an initial period of incapacitation the animal, and presumably man, may be able to perform some tasks for a fleeting period. Single, brief exposures to high doses of radiation will cause profound excitement or convulsions and death within hours or days (70). Although there is little hope of protecting against such lethal exposures, radioprotective agents would be most useful in the lower dose range. It may be possible to use these compounds against sublethal exposures and the

relatively low doses encountered over a period of days or weeks. Chemical protection against these lower doses may be evaluated by animal behavioral tests.

A "behavioral index" has been used to assess the effects of single, relatively low whole-body doses of X-irradiation in rats. The method employs thirsty rats in a chamber monitored to electrically count the number of licks at a water tube. The animals are trained to pattern their water drinking habits (licks) because the drinking time is split into a series of 20-second presentations over a total time of 30 minutes. After a period of time a sustained licking (drinking) pattern is established and the rats are ready for the test situation (71). Following radiation exposure a feeling of illness, discomfort, or some unpleasant sensation presumably ensues in the rat and if the animal is simultaneously presented with a distinctively flavored water to drink, an association develops between the two events. The association of the flavor with the unpleasant sensation (radiation) causes the rat to avoid the flavored fluid. This is an aversive behavioral response. This type of aversive behavior may be used to evaluate radiation protective agents employing saccharine solution as the distinctly flavored fluid taste-cue associated with radiation exposure.

A number of presentations (onset score) is required to establish aversion to the saccharine fluid on the day of irradiation. If the test is repeated on the day after radiation exposure, a "reonset" score is determined as the number of presentations required to reestablish aversive behavior. This reonset score from preliminary experiments was dose dependent in the range of 10 to 50 rad. After 50 rad the reonset score was essentially zero; the rat immediately rejected the saccharine fluid on the day after radiation. Lower radiation doses gave higher scores, i.e., several presentations were required to reestablish aversion. Early findings with 900 mg per kg of cysteine administered intraperitoneally 10 minutes before 35 R irradiation showed higher reonset scores over control rats. These results suggest that this technique might be used to screen protective compounds for effectiveness against damage from low doses of radiation. Obviously the protective agents may influence

these scores and adequate controls must be included.

Animal behavior tests of this type have advantages and disadvantages. Only certain animals develop a stabilized drinking pattern, for example, in the aversive behavior test, and the development of trained animals requires one to two weeks. However, behavior is an extremely sensitive feature of the biological organism and reflects many facets of the life process. Studies designed to quantify these behavioral changes as modified in any way by various kinds of radiation should be extremely useful in elucidating biological effects and also in screening protective compounds.

Radiation behavioral effects are not necessarily mediated through the central nervous system. The association of the radiation "experience" by the rat may be via taste, olfaction (69, 72, 73), or possibly through humoral changes as yet undefined. These taste aversion responses in the rat can be obtained by irradiation of the tail, or abdomen only, with the remainder of the body shielded. It has been suggested that the transfer of the radiation effect may be by some blood element in these latter experiments. Several other reports have suggested that some unidentified humoral receptor system might serve to signal radiation exposure to the test animal (74).

Behavioral studies that measure performance of the animal should include tests that assess motor, sensory, and cognitive skills that may be impaired by sublethal, lethal, or supralethal whole-body doses of radiation (70, 75, 76, 77). General activity of mice is reduced by median doses of 100 rad. On the other hand, arousal responses are the most sensitive measure of behavior to radiation exposure. There is a need to extend behavioral studies to all types of low dose radiation to detect radiation-induced biological effects and to explore these tests as screening measures for protective compounds.

Chromosome Aberrations. Chromosome aberrations induced by relatively low doses of radiation have been used as an index to measure the effectiveness of protective agents

(78, 79, 80, 81). Protection was afforded by cysteine, cystamine, MEA, 5-HT, GSH, and AET. Chromosomes from various cells were examined in these studies and the scoring techniques are similar to those employed in measuring the degree of radiation injury in man (5). These methods are elaborate and require considerable skill but they are sensitive to low doses of radiation and can be quantified. The chemical protective effects extend to cellular processes as reflected in chromosome changes, and the protection suggested resembles hypoxia (81). Both hypoxia and cysteine, for example, increased radiation resistance and hypoxia alone will reduce the number of chromosome breaks produced in mice exposed to 200 R. The combined effect of hypoxia and cysteine was most marked in reducing chromosome aberrations. Additional studies in this area may develop better test measures for use in animals and man subject to various doses of radiation, and to improved techniques for scoring chromosome aberrations.

D. RESEARCH ORIENTATION

At the cellular level the molecular mechanisms involved in repair of injury and division of the cell are of interest in the study of the acute and late effects of radiation. These molecular phenomena may be most radiosensitive and hence become the controlling factor in radiation injury (7). However, mitotic delay or failure, from a number of causes, may follow metabolic changes induced by radiation. The present evidence strongly favors the hypothesis that damage to DNA is a primary cause of cellular radiation injury. Several normal cell divisions may take place before mitosis is inhibited if the radiation dose is low (7). Future radiation protective agents might be designed to specifically protect DNA or encourage DNA repair if the factors influencing the mitotic failure were understood (82). Recovery from radiation injury appears to be an intracellular event.

Future research should include the study of the fine changes in macromolecular protein structures of the mammalian cell. The techniques of physical chemistry have been applied to protein conformation analyses (61, 83). Some changes in the geometric configuration of macromolecular structures induced by radiation are known (84, 85, 86, 87). The precise meaning and correlations of these findings are unknown and therefore interpretation is difficult.

Lacking an understanding of the basic mechanisms of radiation injury, it is difficult to develop working hypotheses for the synthesis of improved radioprotective agents that modify the biological effects of radiation. For this reason, screening programs will continue to exploit the several existing opportunities. However, this process is expensive, time-consuming, and involves a considerable measure of chance. In order to discover "leads" empirical screening requires thousands of compounds before one is found suitable for use in man. Unfortunately, at the present time, there is little evidence that agents developed by these screening techniques would be useful in protecting against doses of radiation that are of greatest interest in the radiological health program.

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IX. AD HOC STUDY GROUP AGENDA

The agenda for the ad hoc study group meeting held at Beaumont, Federation of American Societies for Experimental Biology, Bethesda, Maryland, on March 24 and 25, 1969, included the following topics:

- Chemical Classes, Structures, and Properties
- Mechanisms of Action at the Molecular, Cellular, and Tissue Level
- Comparative Biological Effectiveness
- Pharmacology and Toxicology
- Radiological Factors Influencing Effectiveness
- Screening, Testing, and Evaluating Methods

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X. LIST OF ATTENDEES

AD HOC STUDY GROUP MEETING, MARCH 24 AND 25, 1969

ON

A STUDY OF RADIOLOGICAL HEALTH ASPECTS OF AGENTS MODIFYING THE BIOLOGICAL EFFECTS OF RADIATION

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